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Dynamics of Dental plaque Hygienic index modified by Turesky, Alpha-defensin and Rheological Status estimation in Patients Undergoing Orthodontic Treatment

Tamasidze N.A.
Dental clinic "Archil Tamasidze"
Nikolaishvili M.I.
Prof. and Head of Department
I.Beritashvili Center of Experimental Biomedicine
Mosemghvdlishvili N.G.
Chikobava S.R.
Gogiberidze M.A.
Tamasidze A.G.
Dental clinic "Archil Tamasidze"

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Abstract

Thus, the index of dental plaque was reliably low in patients undergoing the treatment by aligners as compared with the patients with fixed bracket-systems. Non-removable orthodontic equipment affects marginal periodontal tissues and mouth hygienic condition more than removable one. In spite of observed considerable data spread, when comparing the initial level of antimicrobial peptide measured before the placing the bracket systems, statistically significant differences were obtained. In mixed saliva in patients of basic group the content level of alpha-defensin was higher than in the control group. 3 months after carrying bracket systems value index significantly changed: in the group with inflammatory processes the content of alpha defensin decreases in periodontal tissues whilst in comparison group it increases. The changes in basic group after three months was significant. Thus data evaluation suggests that orthopedic problems are accompanied by blood rheological changes that lead to the increase in plasma viscosity red blood cells aggregability and decrease in red blood cells deformation. That is, (blood rheology) and coagulation changes are notable while dysfunction of vascular endothelium is present. These patients might be considered as risk-groups for cardiovascular problems.

Keywords: Alpha-defense, Rheological Status, Orthodontic Treatment.

1. Introduction

Antimicrobial peptides have unique properties: They selectively influence on bacteria, since their cationic molecules have high affinity to the bacterial membrane enriched with negatively charged components-lipopolysaccharides (LPS) and so on. The development of bacterial resistance towards peptides is complicated in connection with the features of the mechanism of its bactericidal action - rapid growth of membrane permeability of microorganism, loss of their barrier function, leading to osmotic the destruction of cells (Nikolaishvili M.I.,2014., Aleshin, G.M., 2010). Thus, the deficiency of antibacterial peptides predisposes to the onset and development inflammatory diseases of pariodentium. (Brill, E.A.2005, Roitman, E.V., 2001)

At present, about a hundred antimicrobial peptides are studied that are detected in epithelial barrier tissues, they phagocytize in cells and human biological fluids, the special place among which have the peptides of oral liquid –defensins (Iordanishvili, A.K., 2007). It is estimated that the patients, who lack for alpha-defensins, suffer from common and severe bacterial infections.

The application of antimicrobial peptides as diagnostic markers for the prevention of periodontal tissues from bacterial aggression is gaining more and more importance. The systematic review of the literature regarding the application of antimicrobial peptides against pathogens of oral cavity was carried out. The researchers detected that the peptides, produced by oral mucosal membrane (defensins, LL-37 and histatins) and Porphyromonas gingivitis were sufficiently studied. They introduced the data showing that antimicrobial peptides could affect the development of periodontal tissues diseases by inactivation of bacterial or host-protease, or bind bacterial toxins, including lipoplysaccharides (e.g. LL-37) where the role of LL-37 is also shown in the development of inflammatory diseases of periodontal tissues. In the work the general knowledge upon beta-defensins is also considered and new aspects emphasizing their important role in the destruction of periodontal tissues are introduced. On the whole, the discovery discussed in the literature considerably expands the modern ideas about the role of antimicrobial peptides in the mechanisms of specific and nonspecific prevention of periodontal tissues and opens new opportunities in the prevention and treatment of the disease.

All the above suggest that in the resent years, thanks to the development of clinical immunology, the attention of researchers has been drawn by immunological aspects of periodontal diseases. The destruction of the tissue integrity in periodontal mechanisms was attributed to immune depressive

diseases. (Brill, E.A, 2005, Mockovcki, A.V., 2010). However, there is no consensus regarding the approaches to the estimation of risk factors at the development of pathological process in periodontal tissues and its correlation to local mechanisms of immune reactivity of oral cavity (Wahab, R.M., 2012). In consideration of the complexity of immunological response of human body, it is almost impossible to consider any one inflammatory mediator as diagnostic marker of the disease.

There are only fragmentary data in the literature upon the features of the immunity during orthodontic treatment with bracket system (Sushkova M.A., 2002). In connection with it the study of the parameters of cellular and humoral links of the immunity in orthodontic patients is quite topical. The detection of the periods of immunological tension at the stages of orthodontic treatment will enable to develop qualitatively new approach to the prevention of periodontitis in these patients.

2.Materials and study methods

In order to estimate hygiene, we applied the index of dental patch modified by Turesky (table 1). The index of dental patch modified by Turesky (1970) (Turesky S, 1970). This index is the same as the Quigley Hein Index except the criteria has been modified. As Quigley Hein Index, a score of 0 to 5 is assigned to each facial and lingual nonrestored surface of all the teeth except third molars in both groups was estimated as follows: 0.75% the main fuchsine dye solution was applied to dry through vestibular and lingual surface of all groups of teeth, the patient rinsed the mouth with ordinal water following which the amount of dental patch on the specified surfaces was estimated by the following scale:

0 score: lack of plaque

1score –solitary patch in cervical part of the tooth

2 scores—thin uninterrupted strip of plaques (up to 1mm) in cervical part of the tooth

3 scores-- strip of plaques wider than 1mm, although covers less than 1/3 crown of tooth.

4 scores—the plaque covers more than 1/3 but less than 2/3 crown of tooth.

5 scores- the plaque covers 2/3 crown of tooth and more

Turesky index was evaluated as follows: PI =dental plaque value/2xn, Where n is the quantity of teeth.

Figure 5. An index for the entire mouth is determined by dividing the total score by the number surfaces (a maximum of 2 x 2 x 14 = 56 surfaces) examined

As a result of the analysis of clinical material on the 3rd month of orthodontic treatment using fixed equipment, the patients were ranked on two groups : the first (main) included the patients, where in the treatment process of dentoalveolar pathologies using fixed orthodontic equipment, some inflammatory processes arouse in periodontal tissue, whilst the second group (control group) included the patients who did not have any signs of inflammation in periodontal tissue over the entire orthodontic treatment.

In order to study the local immunity of the oral cavity of patients with dento-alveolar pathologies, the oral saliva was examined in patients with teeth-jaws anomalies. The material was unstimulated mixed saliva.

Before collecting the samples the patients were offered to rinse the mouth with boiled water at room temperature. Neither somatic diseases had been indicated, nor pharmacotherapy was applied to the patients participating in the study. For the collection of the samples , sterile vials were used in each case. The saliva was subjected to centrifugation at 300 rpm for 15 minutes, and then refrigerated at -70°C.

The concentration of alpha-defensin was measured using HBT ELISA TEST KITS FOR HNP 1-3, meant for quantitative measurement of defensines neutrophiles in mixed saliva (See Fig. 2)

Fig 2--- reagents kit for the measurement of alpha-defensine 1-3 by immune-enzyme assay

The principle of HBT NPH 1-3 ELISA is based on "sandwich" method of enzyme-linked immunsorbant ***assay*** (ELISA technique). Samples and standards were incubated in microplate holes covered by antibodies toward human alpha-defensine. biotin-conjugated antibodies (feeler) bind to human HNP1-3. The conjugate of streptavidin-peroxidase binds to the feeler. The conjugate of streptavidin-peroxidase interacts tertramethylbenzidine substrate (TMB). Absorbance at 450 nm is measured by spectrophotometer. Calibration (standard) curve is obtained by the schedule of dependence of optical density from the corresponding concentration of the defensine of neutrophils.

Blood rheology status was thoroughly measured: 1. Red blood cells concentration –local hematocrit. 2. Red blood cells membrane deformability, that is particularly important in brain microvessels where the diameter of capillaries is the least and red blood cells experience strong deformation in this microcirculation. 3. Red blood cells aggregation 4. Plasma viscosity. Red blood aggregation index was measured by the ratio of red blood cells aggregation index that is aggregated red blood cells area to the whole area of red blood cells by so called "Georgian technique". Red blood cells deformability index will be measured by ucleopore membrane filter method that is based on the measurement of red blood cells flow rate in the thinnest capillary 5um pore filter under constant pressure (10 cm water column) . Plasma viscosity is measured in 37^0C capillary viscometer. Systemic hematocrit is measured by blood sample centrifugation G-3500, median radius 5 cm, 3000 r/sec for 10 minutes. Plasma viscosity is measured at 37^0C in the capillary viscometer. Capillary diameter is 1.8 mm. Plasma flow in blood vessels is induced by the force of gravity which is determined by the difference of the studied plasma, about 65 mm.

3. Results

One of the most important tasks of the present study was the measurement of the influence of aligners on the emergence of compound cavity that actively emerges in course of orthodontic treatment (Mantskava, M.,2014, Roitman , E.V. 2001,Katyuhin L.N. 1995). Therefore the onset of white carious spots was separately taken into consideration. In order to estimate the hygiene level the index of dental patch modified by Turseky was used (table 1).

Table 1. Dynamics of hygienic index modified by Turseky in the process of orthodontic treatment using aligners and bracket systems

Turseky index (M±*m)*						
	Before treatemnt	1 month	3 month	6 month	9 month	12 month
brackets	1,25±0,65	1,75±0,23	2,28±*0,29*	2,32±*0,24*	2,47±*0,32*	2,42±*0,37*

aligners	1,03±*0,23*	1,22±*0,1*	1,20±*0,12*	1,19±*0,14*	1,2±*0,16*	1,11±*0,1*
N	52	52	52	52	52	52
T_{fact}	0.25	0,11	3,02	3,39	3,01	2,002
$T_{kh(0,0,5;56)}$	2,002	2,002	2,002	2,002	2,002	2,002
P	>0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Difference between mean values	Not reliable	Reliability difference	Reliability difference	Reliability difference	Reliability difference	Reliability difference

At the initiation of the treatment hygienic level in patients of both groups was on average 1.03-1.25 with unreliable differences. 1 month later index values increased in both groups: up to 1,22 ± 0,1 in patients with aligners and up to 1,75 ± 0,23 in patients with bracket-systems. In the patients with aligners, the basic foci of accumulation of dental patch were detected in the area of composite attachments. At the beginning of the 3rd month the index value was stabilized at 1,20±0,12 level in patients who were treated by aligners. In the patients treated with brackets systems it reached 2,28 ± 0,29 that could be explained by the emergence of various elastic recoils at this stage also promoting the accumulation of dental patch. In the course of further observation, the further tendency to the increase of index value was detected in the patients with bracket systems within a year up to 2.42-2.47 versus stabile index 1.11-1.2 in patients with aligners. Under conditions of the existence of dental patch (that was available according to data by Turseky index) there were favorable conditions for breeding of pathogenic bacteria and development of carious lesions. The basic accumulation of dental patch was detected in the area of lateral incisors as well as around fixation. Thus, dental patch index was reliably low in patients treated with aligners, versus the patients treated with bracket systems. Fixed orthodontic equipment has more unfavorable influence on marginal periodontal tissue and hygienic condition of the oral cavity than removable ones (Womack, W.R., 2008, Wong, B., 2002).

At present the study of antimicrobial peptides providing the realization of protective and adaptive response of the body at infections and stress load, the content of which was measured in mixed saliva in orthodontic patients is of great interest (Chichinadze K., 2014,Wahab, R.M., 2012, Womack, W.R., 2008). Antimicrobial peptides are themselves small cationic peptides, influencing microorganisms through the deterioration of membrane permeability forming ion channels.

Three main human defensins (HNP 1-3) can probably be set at 99% of all antimicrobial peptides of this type. They are synthesized only by neutrophils that enables to consider them specific cellular markers of these cells. Neutrophil activation during acute infection or inflammation leads to

rapid release of defensins that are later detected in plasma and other body fluids.

In addition of microbicidial action, Alpha-DH also has chemostatic, immunomodulation, and cytotoxic activities, contribute to immunological protection of the body and the development of inflammatory processes.
Level index of the content of alpha defensin in mixed saliva in the patients of main group and comparison groups are given in table 2.

Table 2. The content of alpha defensin (ng/ml) in mixed saliva in the patients of main group and comparison groups on different terms of orthodontic treatment

Period of index measurement	Main group n=54		Comparison group n=43		Significance level m/y according to groups , p
	min-max	*M±m*	*min-max*	*M±m*	
Before placing bracket-system	12 – 1365	469,0±55,7	0 – 1813	297,1±57,7	<0,05
3 months after orthodontic treatment.	18 – 769	333,5±31,6	5 – 1096	408,7±53,9	>0,05

In spite of observed considerable data spread, when comparing the initial level of antimicrobial peptide measured before the placing bracket systems, statistically significant differences ($p<0.05$) were obtained. In mixed saliva in patients of basic group the content level of alpha-defensin was higher 469,0±55,7 пг/мл than in comparison group 297,1±57,7 пг/мл. 3 month after carrying bracket systems value index significantly changed: in the group with inflammatory processes the content of alpha defensin decreases in periodontal tissues up to 333,5±31,6 пг/ml whilst in comparison group it increases up to 408,7±53,9 пг/мл . the changes in basic group in three month – significant ($P<0.05$).

The further investigation was conducted regarding blood theology . As it is known from the literature (Krupatkin, A.I.2005, Azizova O.A.,2001) in patients undergoing orthodontic treatment inflammatory processes develop, that are very considerable as they lead to cardiovascular diseases risk and there are some data according to which the development of periodontitis is associated with significant atherosclerotic disorders of large caliber arteries (Wong, B., 2001). Therefore the topic is topical and extremely significant, that's why we decided to study blood rheological components in patients undergoing orthodontic treatment. (See Tabl.3).

Table 3. Hemorheologic status (red blood cells aggregability, red blood cells deformability, hematocrit, plasma viscosity) in clinical investigation patients

	Red blood cells aggregability, EAI		Red blood cells deformability, EDI		hematocritHCT		Plasma viscosity Y Puisi 10 din x cm/cm^2	
	Basic group	Control group	Basic group	Control group	Basic group	Control group	Basic group	Control group
Before placing bracket -system	26,7±0,21	24,7±0,01	2,20±0,01	2,18±0,01	44,2±0,23	42,3±0,21	1,12±0,01	1,12±0,01
3 months after orthodontictreatment	30,6±0,12	25,2±0,11	2,22±0,01	2,19±0,01	46,5±0,32	41,2±0,12	1,31±0,02	1,14±0,01
3 months after aligners	22,6±0,03	20,2±0,12	2,17±0,01	2,14±0,01	39,5±0,32	41,2±0,12	1,16±0,02	1,13±0,01

4. **Conclusion**

Thus data evaluation suggests that orthopedic problems are accompanied by blood rheological changes that lead to the increase in plasma viscosity and red blood cells aggregability. That is, blood rheology and coagulation changes are notable while there is dysfunction of vascular endothelium. And this is a very significant problem. These patients might be considered as risk-groups for cardiovascular problems. Study measures of rheological parameters have not been implemented in dental clinics of Georgia yet. With this work we would like to show how important theological changes are. At the time we would like to show that aligners are very convenient, they are also very beautiful, although non-removable brackets have their strong side, when it is necessary to straighten teeth and their insertion in appropriate place and stretching becomes necessary in certain conditions. Brackets are unchangeable in this situation. Of course a doctor must be trained and a patient should maintain personal hygiene.

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